Journal of Medical Virology 49:218–222 (1996)

Evaluation of a Pooling Method for Routine Anti-HCV Screening of Blood Donors to Lower the Cost Burden on Blood Banks in Countries Under Development

Zaida García, Lizeth Taylor, Alcira Ruano, Lizeth Pavón, Esperanza Ayerdis, Ronald B. Luftig, and Kirsten A. Visoná

Louisiana State University-International Center for Medical Research and Training (Z.G., L.T., K.A.V., Costa Rica, R.B.L., New Orleans,) and Red Cross Blood Banks from El Salvador, Honduras, and Nicaragua (A.R., L.P., E.A.), Louisiana State University-International Center for Medical Research and Training, San José, Costa Rica

A pooling system was developed for use in anti-HCV screening of voluntary blood donors at the local Central American Red Cross blood banks, in Nicaragua, El Salvador and Honduras. The commercially available second generation anti-HCV screening kit from Abbott Laboratories (North Chicago, IL) was used with a modification in the initial serum dilution procedure. Pools of five sera were selected for routine screening, based on comparative studies of individual samples and of pools with different sample sizes.

During the years 1993 and 1994 a total of 89,148 voluntary blood donors were screened and a positive prevalence rate of 0.35% was established. Of the initially positive samples, 54% confirmed positive, 30% were indeterminate and 16% were negative using the Abbott Matrix test.

Significant differences of positive screening prevalence rates were found in the three countries, with average values of 0.50%, 0.23% and 0.08%, respectively, in Nicaragua, El Salvador and Honduras. These initially positive samples also showed a different confirmatory pattern with a positive rate of 64% in Nicaragua, in contrast to 20% in El Salvador. Only a few samples were available for RT-PCR amplification of HCV-RNA; however, this highly sensitive method did not appear to be more helpful than serology in confirming the HCV donor status.

Overall, the data obtained indicate a fluctuation of HCV prevalence in voluntary blood donors among the three Central American countries. Further, differences were found in the percentages of initially screened positives and confirmation patterns. This information appears useful for establishing criteria in future screening policies. Thus, we suggest that the use of pooling for anti-HCV screening is beneficial in countries under development, since there are potential cost savings, as well as benefits in establishment of initial prevalence rates.

KEY WORDS: hepatitis C, Central America, confirmatory results, specific epitopes

INTRODUCTION

Hepatitis C virus (HCV) is the major cause of post-transfusion acquired hepatitis [Garson et al., 1990; Aach et al., 1991]. In developed countries, routine screening of blood donors for anti-HCV was implemented half a decade ago; however, in most developing countries these screening tests are excluded because of their high cost. Unfortunately, many of these countries have high prevalence rates [Vanderborght et al., 1993; Visoná, 1993; Martins et al., 1994].

The risk of post-transfusion HCV infection in single-donor products fluctuated from 1% to >15% throughout the world before implementation of anti-HCV blood donor screening and was closely related to the prevalence rate of a given donor population [Alter and Seeff, 1993]. Polytransfused patients such as hemophiliacs and those on hemodialysis were at an even higher risk [Brettler et al., 1990; Blanchette et al., 1991; Peters et al., 1993; Allander et al., 1994; Mauser-Bunschoten et al., 1995]. In countries where the routine screening of anti-HCV in blood donors has been adopted, the risk of acquiring HCV post-transfusion infection has been reduced to <0.1% [Esteban et al., 1991; Okochi et al., 1991; Nelson et al., 1992].

Accepted for publiation February 24, 1996.

Address reprint requests to Dr. Kirsten A. Visoná, Louisiana State University-International Center for Medical Research and Training, Jet Box-Miami, 025240, Miami, FL 33102-5240. Cuenta SJO 321.

TABLE I. Protocol of Sera Dilution to Be Used for Anti-HCV EIA

	μl of serum	μl of diluent	Dilution factor
Individual sera	10	400	1:40
Pool of five	4 each	200	1:50
Pool of eight	3 each	200	1:64
Pool of ten	2 each	200	1:100

The aim of this study was to develop a pooling test to be used for routine anti-HCV screening of blood donors, where cost is the limiting factor for its implementation. This has previously been recommended for HIV screening (WHO AIDS Meeting in San Jose, Costa Rica, 1992). In the current HCV study, important data on prevalence were obtained, which aids in understanding the complex nature of HCV infection in Central America.

MATERIALS AND METHODS

Study Population

In order to adequately develop an anti-HCV pooling system, 2,480 voluntary blood donors and 208 post-transfusion patients from three Central American countries were enrolled during 1992. Sera from these individuals were initially screened by EIA anti-HCV as single samples, followed by retesting in pools of 5, 8 and 10 samples. The results of this study were used to optimize the pooling system.

During the years 1993 and 1994, a total of 89,148 sera samples of voluntary blood donors from the Red Cross blood banks in Honduras, Nicaragua and El Salvador were screened for anti-HCV by EIA, using pools of five samples. Antibody positive pools were retested as individual samples. All positive sera were confirmed by the Abbott Matrix System. Eighteen randomly selected samples from Nicaragua were tested by RT-PCR for HCV-RNA.

Screening Assay

For anti-HCV detection an EIA second generation screening kit from Abbott Laboratories was used [Alter, 1992; Anderson et al., 1995]. The manufacturer's instructions were followed for the testing of individual sera. In adapting this assay to the pooling system, only the initial dilution procedure was changed as shown in Table I. The cut-off optical density (OD) value was calculated as described by the manufacturer (Abbott Laboratories, North Chicago, IL), in both the setting of individual serum and for the pooling system.

Confirmatory Assay

All positive samples by EIA screening were re-analyzed by the Abbott Matrix assay [Tibbs et al., 1991; Chaudhary et al., 1994]. This test is based on detection of specific antibodies reacting with three different epitopes of HCV: core, NS3 and NS4. The NS4 epitope was included as two separate antigens, NS4(y) and NS4(e).

TABLE II. Results of Confirmation Testing* of Anti-HCV Sera and Their Detectability in a Screening Assay of Pooled Sera

Confirmed results of individual sera[1]	Total	Results of Anti-HCV: Pool of five sera Pos.	Pool of five sera Neg.	Pool of ten sera Pos.	Pool of ten sera Neg.
Positive	38	37	1	36	2
Indeterminate	12	4	8	3	9
Negative	6	0	6	0	6

*Abbott Matrix system was used for confirmation tests.
[1]All are initially positive screened sera.

TABLE III. Results of Anti-HCV Prevalence in Blood Donors Screened by the "Pool of 5" Method 1993–1994

Country	Year	Anti-HCV screening: Total	Pos. (%)
Nicaragua	1993[1]	22945	101 (0.44)
	1994	24336	139 (0.57)
El Salvador	1993	15073	37 (0.25)
	1994	13453	30 (0.22)
Honduras			
Tegucigalpa	1993	4427	5 (0.11)
	1994	5377	5 (0.09)
San Pedro Sula	1993	1896	0 (0.00)
	1994	1641	0 (0.00)
Total		89148	317 (0.35%)

[1]Jan. 1–Nov. 1.

RT-PCR for HCV-RNA

RNA was extracted from 100 μl of serum with an RNeasy spin column (Qiagen, Chatsworth, CA), and the cDNA was prepared using Super Script II RT (Gibco, Grand Island, NY) and oligo-dT. The region to be amplified by PCR corresponded to the 5′ NCR. A method of nested PCR with "hot-start" was utilized with Taq polymerase (Perkin Elmer, Foster City, CA). The primers for the first PCR were HC1 (5′-GGCGACACTCCACCATAGATC-3′) and HC2 (5′-CATGGTGCACGGTCTACGAGACC-3′) while for the nested PCR HC3 (5′-GGAACTACTGTCTTCACGCACA-3′) and HC4 (5′-TCGCAAGCACCCTATCAGGCAG-3′) were used. The PCR reaction was run for 30 and 40 cycles, respectively using a 9600 Perkin Elmer thermocycler. For the initial PCR each 30 sec cycle was at 95°C, 56°C, 60°C and 72°C. For the nested PCR each 30 sec cycle was at 94°C, 60°C, 64°C and 72°C. The final PCR product of 173 bp was analyzed on agarose gel stained with ethidium bromide and/or SYBR Green I [Widell et al., 1991].

RESULTS

A comparison of confirmatory tests among initially positive screened individual sera and pools of five and 10 samples is shown in Table II. The "pools of five" system, exhibited an enhanced sensitivity, i.e. 97% vs.

TABLE IV. Pattern of Anti-HCV Confirmatory Testing in Positive Screened Samples From Voluntary Blood Donors

Country	Date	Total	Pos. (%)	Ind. (%)	Neg. (%)
Nicaragua	Jan. 93–Jan. 95	302	194 (64)	74 (25)	34 (11)
El Salvador	Jan. 93–Jan. 95	89	18 (20)	43 (48)	28 (31)
Honduras	Jan. 93–Jan. 95	1	5/11	4/11	2/11

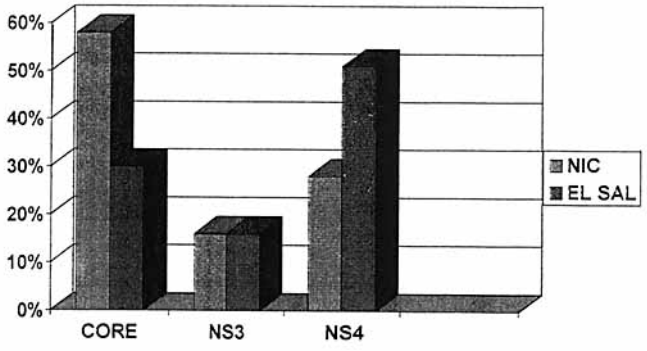


Fig. 1. Results of indeterminate samples reactivity against HCV specific epitopes.

TABLE V. Relationship Between Specific Antibodies to Different HCV Epitopes for Indeterminate Sera Obtained From Screening of Individual and Pooled Sera

Year	Screening system	Total	Core	NS3	NS4
1992	"Individual sera"	12	5	2	5
1993	"Pools of five"	20	8	3	9

95%. Pools of five and eight gave the same percentage. However, a lower overall OD reading was found in pools of eight, as well as pools of 10 sera in comparison with the pools of five, increasing the possibility of obtaining false negative results. Thus, use of the larger pools was discarded as a suitable screening system.

"Pools of five" showed the same specificity as the screen test of individual samples. One undetected (negative) sample in a pool had a low positive ratio when screened as an individual serum sample (ratio 1:3; sample OD/cut-off OD). Five of the eight indeterminate samples found to be negative in the pools reacted only with the NS4 epitope.

Routine screening of blood donors using "pools of five" was started on January 1, 1993. The prevalence rates obtained over the first 2 years are shown in Table III, with average yearly rates of 0.50%, 0.23% and 0.08% in Nicaragua, El Salvador and Honduras, respectively. The confirmatory pattern in Table IV shows a much higher prevalence of positivity in Nicaragua (64%) than in El Salvador (20%). Patterns of indeterminate sera reacting to specific epitopes were compared among the above two countries. Samples from Nicaragua were predominantly reactive with the HCV core epitope (58%) in contrast to those from El Salvador, where the highest reactivity was to NS4 (51%) (Fig. 1). The total number of initially positive samples in Honduras was too low to use for this calculation.

Table V compares the pattern of reactivity to specific HCV epitopes of indeterminate samples from all three countries obtained in the earlier screening system of "individual sera" and "pools of five." A similar pattern was observed in these two groups, suggesting that the composition of initially reactive samples included in the "pools of five" was not biased.

RT-PCR results were obtained from 18 randomly selected samples of Nicaraguan donors (Fig. 2). Three of the 18 samples which were classified as indeterminate by the Matrix test had undetectable levels of HCV-RNA (Fig. 2; lanes 2, 8 and 14). Seven of 13 samples confirmed positive by the Matrix test had a positive RT-PCR result. Two additional undetected samples by RT-PCR had a confirmed negative antibody state.

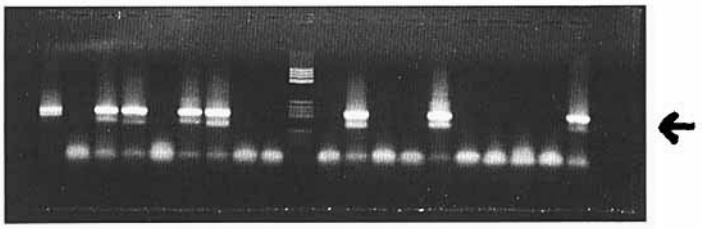
DISCUSSION

The overall anti-HCV prevalence rate of voluntary blood donors in three Central American countries was determined and found to vary from an average of slightly more than 0.5% in Nicaragua to <0.1% in Honduras, using similar criteria for donor selection.

Why do these differences occur? Little is known about the natural history of HCV infection in Central America. The donor populations do not appear to have a history of blood transfusion, since this is a criterion used for donor exclusion. However, some of the Central American countries have a recent history of civil war (Nicaragua and El Salvador) under which circumstances there may have been close contact among injured HCV positive people or possibly common reusing of blood-contaminated material. The higher HCV prevalence rate in Nicaragua could be due to such events. Also, the high rate of indeterminate samples reacting with core epitope suggests that there is an active ongoing infection of HCV in Nicaragua, since antibodies to core alone often indicates the presence of a recent infection [Halfon et al., 1992; Pawlotsky et al., 1994; Yuki et al., 1994].

In contrast, the findings in El Salvador with a twofold lower screening prevalence rate and with most indeterminate samples reactive against the NS4 epitope provide evidence of a different HCV status. Reactivity to the NS4 epitope alone is often caused by cross-reactivity to unrelated viral proteins and a high percentage of such samples have HCV-RNA levels that are reported to be undetectable by PCR [Schwarcz et al., 1990; Theilmann et al., 1990; Chichenportiche et al., 1993; Sakugawa et al., 1995].

Differences in HCV genotypes can also lead to changes



Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Matrix test + I + + - + + I + + + - I + + + - C- C+

Fig. 2. RT-PCR for HCV-RNA of 18 samples from Nicaragua. Lane 10 displays MW markers from a Hae III Digest of pBR322 DNA (Boehringer Mannheim). Arrow points to the 173 bp product, indicative of a positive PCR test. I indicates the indeterminate samples.

in the pattern of reactivity to specific epitopes [Cuypers et al., 1991; Maggi et al., 1995; Pawlotsky et al., 1995]. Since none of the blood donors in this study have been genotyped and only a few samples tested by RT-PCR for HCV-RNA due to logistic problems of transporting samples, further studies are needed in order to better understand the differences observed [Gournay et al., 1995; Yuki et al., 1995]. Additionally, from the results seen in Figure 2, it is clear that RT-PCR does not seem to be a technique that provides further confirmation of HCV status.

Ideally, screening of anti-HCV in blood donors would be to test individual samples. However, due to various reasons <30% of all blood donors were screened for anti-HCV in Central America during 1993 and a similar percentage during 1994 (information from PAHO in documents and meeting held in El Salvador, July 1994). The pooling method described above using "pools of five" offers an alternative to more costly diagnostic tests for routine testing of anti-HCV in blood donors. An overall analysis for the 2 years of pool-based screening has shown savings of about 75% in cost of reagents as compared to using individual sera tests (K. Visoná, unpublished data). Further, the use of pooling has been helpful in generating prevalence rates which can be used in the future to establish screening policies.

ACKNOWLEDGMENTS

The authors thank Dr. Sally Hojvat from the Abbott Laboratories (North Chicago, Chicago, IL) for facilitating the confirmatory testing and to Ms. Virginia Larrad for editorial support.

REFERENCES

Aach RD, Stevens CE, Hollinger FB, Mosley JW, Peterson DA, Taylor PE, Johnson RG, Barbosa LH, Nemo GJ (1991): Hepatitis C virus infection in post-transfusion hepatitis. New England Journal of Medicine 325:1325–1329.

Alter HJ (1992): New kit on the block. Evaluation of second generation assay for detection of antibody to the hepatitis C virus. Hepatology 15:350–352.

Alter HJ, Seeff LB (1993): Transfusion-associated hepatitis. In Zuckerman Thomas HC (eds): "Viral Hepatitis. Scientific Basis and Clinical Management." Edinburgh, London, Madrid, Melbourne, New York, Tokyo: Churchill Livingstone, pp 467–500.

Allander T, Medin C, Jacobson SH, Grillner L, Persson MAA (1994): Hepatitis C transmission in a hemodialysis unit: Molecular evidence for spread of virus among patients not sharing equipment. Journal of Medical Virology 43:415–419.

Anderson SC, Hathaway T, Kuramoto IK, Holland PV, Gilcher R, Koch T, Hojvat S (1995): Comparison of two second-generation anti-hepatitis C virus ELISA on 21431 US blood donor samples. Journal of Viral Hepatitis 2:55–61.

Blanchette VS, Vortsman E, Shore A, Wang E, Petric M, Jett BW, Alter HJ (1991): Hepatitis C infection in children with hemophilia A and B. Blood 78:285–289.

Brettler DB, Alter HJ, Dienstag JL, Forsberg AD, Levine PH (1990): Prevalence of hepatitis C virus antibody in a cohort of hemophilia patients. Blood 76:254–256.

Cuypers HTM, Winkel IN, van der Poel CL (1991): Analysis of genomic variability of hepatitis C virus. Hepatology 13:S15–S19.

Chaudhary RK, Jacobsen H (1994): Performance of third-generation confirmatory tests for detection of antibody to hepatitis C virus. Journal of Clinical Microbiology 32:2606–2608.

Chicheportiche C, Cantaloube JF, Biagini P, Aumont F, Donnadieu J, Escher F, Larabi F, Zepitelli JP (1993): Analysis of ELISA hepatitis C virus-positive blood donor population by polymerase chain reaction and recombinant immunoblot assay (RIBA). Comparison of second and third generation RIBA. Acta Virological 37: 123–131.

Esteban JI, González A, Hernández JM, Madoz P, Muñiz E, Torras J, Enriquez J, Buenestado J, Martín-Vega C, Sánchez C, Esteban R, Guardia J, Houghton M, Alter HJ (1991): Open prospective efficacy trial of anti-HCV screening of blood donors to prevent posttransfusion hepatitis. In Hollinger FB, Lemon SM, Margolis HS (eds): "Viral Hepatitis and Liver Disease." Williams and Wilkins, Baltimore, pp 431–433.

Garson J, Tuke P, Makris M, Briggs M, Machin S, Preston F, Tedder R (1990): Demonstration of viremia patterns in haemophiliacs treated with hepatitis-C-virus contaminated factor VIII concentrates. Lancet 336:1022–1025.

Gournay J, Marcellin P, Martinot-Peignoux M, Degott C, Gabriel F, Courtois F, Branger M, Wild AM, Erlinger S, Benhamou JP (1995): Hepatitis C virus genotypes in French blood donors. Journal of Medical Virology 45:399–404.

Halfon P, Rousseau S, Tamalet C, Antoni M, Gerolami V, Levy M, Bourliere M, Planells R, Cartouzou G (1992): Indeterminate second generation hepatitis C recombinant immunoblot test: Detection of hepatitis C virus infection by polymerase chain reaction. Journal of Infectious Diseases 166:449.

Maggi F, Vatteroni ML, Pistello M, Avio CM, Cecconi N, Panicucci F, Bendinelli M (1995): Serological reactivity and viral genotypes in hepatitis C virus infection. Journal of Clinical Microbiology 33:209–211.

Martins RMB, Vanderborght BOM, Rouzere CD, Santana CL, Santos CO, Mori DN, Ferreira RG, Yoshida CFT (1994): Anti-HCV related to HCV PCR and risk factors analysis in a blood donor population of Central Brazil. Revista do Instituto de Medicina Tropical de Sao Paulo 36:501–506.

Mauser-Bunschoten EP, Bresters D, van Drimmelen AAJ, Roosendaal G, Cuypers HTM, Reesink HW, van der Poel CL, van den Berg

HM, Lelie PN (1995): Hepatitis C infection and viremia in Dutch hemophilia patients. Journal of Medical Virology 45:241–246.

Nelson KE, Donahue JC, Munoz A, Ness PM, Yawn D, McAllister H (1992): The risk of transfusion transmission of hepatitis C virus (HCV) and effectiveness of donor screening. Abstracts of the 5th National Forum on AIDS, Hepatitis and other Blood-Borne Diseases, Atlanta, GA, p 70.

Okochi K, Inaba S, Tokunaga K, Yoshizawa H, Moori H, Mizui M, Ito S, Yokoishi F, Tsubaki K, Yamano H, Konishi K, Kanemitsu K, Shimizo M, Watanabe J, Nojiri N, Nishioka K, Tanaka T, Kikuchi S, Endo N (1991): Effect of screening for hepatitis C virus antibody and hepatitis C core antibody on incidence of post-transfusion hepatitis. Lancet 338:1040–1041.

Pawlotsky JM, Fleury A, Choukroun V, Deforges L, Roudot-Thoraval F, Aumont P, Duval J, Dhumeaux D (1994): Significance of highly positive c22-3 "Indeterminate" second generation hepatitis C virus (HCV) Recombinant Immunoblot assay (RIBA) and resolution by third-generation HCV RIBA. Journal of Clinical Microbiology 32:1357–1359.

Pawlotsky JM, Roudot-Thoraval F, Pellet C, Aumont P, Darthuy F, Remire J, Duval J, Dhumeaux D (1995): Influence of hepatitis C virus (HCV) genotypes on HCV Recombinant Immunoblot Assay patterns. Journal of Clinical Microbiology 33:1357–1359.

Peters T, Schlayer HJ, Preisler S, Kopp B, Berthold H, Gerok W, Rasenak W (1993): Frequency in hepatitis C in acute post-transfusion hepatitis after open-heart surgery. A prospective study in 1500 patients. Journal of Medical Virology 39:139–145.

Schwarcz R, Weiland O, Sydow M (1990): False positive reactivity for antibodies against hepatitis C virus in patients with autoimmune chronic active hepatitis? Scandinavian Journal of Infectious Diseases 22:377–378.

Theilmann L, Blazek M, Goeser T, Gmelin K, Kommerell B, Fiehn W (1990): False-positive anti-HCV tests in rheumatoid arthritis. Lancet June 2:1346.

Tibbs CJ, Palmer SJ, Clark CSK, Parsons GM, Hojvat S, Peterson D, Banatvala JE (1991): Prevalence of hepatitis C in tropical communities: The importance of confirmatory assays. Journal of Medical Virology 34:143–147.

Sakugawa H, Nakasone H, Nakayoshi T, Kinjo F, Saito A, Yakabi S, Zuckeran H, Miyagi Y, Taira R, Koja K, Uezu T, Kina M, Omine K (1995): High proportion of false positive reactions among donors with anti-HCV antibodies in a low prevalence area. Journal of Medical Virology 46:334–338.

Vanderborght BOM, Reis AMM, Rouzere CD, Salgado da Silva R, Yoshida CFT, Franco LGP, Maertens G, Van Heuverswijn H, Moreira Pereira J (1993): Prevalence of anti-hepatitis C virus in the blood donor population of Rio de Janeiro. Vox Sanguinis (Basel) 65:122–125.

Visoná KA (1993): Evaluation of blood-transmitted infectious agents in blood donors in Central America. Final Report. Submitted to the International Federation of Red Cross and Red Crescent Societies, Basel, Switzerland.

Widell A, Mansson A-S, Sundstrom G, Hansson BG, Nordenfelt E (1991): Hepatitis C virus RNA in blood donor sera detected by the Polymerase Chain Reaction: Comparison with supplementary hepatitis C antibody assays. Journal of Medical Virology 35: 253–258.

Yuki N, Hayashi N, Kasahara A, Hagiwara H, Ohkawa K, Fusamoto H, Kamada T (1994): Hepatitis C virus replication and antibody responses toward specific hepatitis C virus proteins. Hepatology 19:1360–1365.

Yuki N, Hayashi N, Mita E, Hagiwara H, Oshita M, Ohkawa K, Katayama K, Kasahara A, Fusamoto H, Kamada T (1995): Clinical characteristics and antibody profiles of chronic hepatitis C patients: Relation to hepatitis virus genotypes. Journal of Medical Virology 45:162–167.